# The Conservation Agency

*Exploration, Education, and Research*

*President*
*James D. Lazell, Ph.D.*
*401-423-2652*

*6 Swinburne Street*
*Conanicut Island*
*R.I. 02835 U.S.A.*

21.viii.07

Hi Henry,

Herewith our first significant DNA MS, submitted initially to the top journal in herpetology, Copeia. It may not be important enough for them. Worth a try....

Several more are nearing completion: two on skinks (only one relevant to Guana), and one on iguanas (very relevant to Guana!).

DNA work takes time!

Thanks for your support! Hope we can get more this year.

All the best,

Skip

**Title:**

Population Disjunction and Relationships of Some House Geckos in *Gekko* and *Hemidactylus* (Squamata: Gekkonidae) Inferred from 12S rRNA of Mitochondrial DNA

**Authors and Affiliations:**

Ning Qing

Ranran Tong

South China Normal University, College of Life Science, Guangzhou, Guangdong 510631, China. Phone: 20 8521 1375. Email: qingn@scnu.edu.cn

Wenhua Lu

James Lazell

The Conservation Agency, 6 Swinburne Street, Jamestown, RI 02835, USA. Phone 401 423 2652. Email: hq@theconservationagency.org

**Corresponding Author:** Ning Qing, South China Normal University, College of Life Science, Guangzhou, Guangdong 510631, China. Email: qingn@scnu.edu.cn

**Running Head:** GECKO MITOCHONDRIAL DNA

**Manuscript Type:** Shorter Contribution

**Key Words:** Austro-boreal disjunction, Nan Ao Island, South China, genetic distance

**The status of reptiles and amphibians on the South China Sea island of Nan Ao is of great interest because the island harbors populations that often appear to be disjunct from the main ranges of their species farther north, inland and upland. The intermediate zone between the disjuncts may be inhabited by different (often tropical) species of similar ecological niche. Sequencing the 12S rRNA segment of mitochondrial DNA upholds the hypothesis of austro-boreal disjunction in *Gekko subpalmatus* between populations from Nan Ao Island and interior upland China. They are conspecific, as are populations of *G. chinensis* on a tropical Hong Kong island and the adjacent mainland in the intermediate zone between the *G. subpalmatus* disjuncts. The degree of difference in genetic distance between *G. subpalmatus* and its parapatric congener *G. chinensis* is greater than that between *Hemidactylus bowringi* and sympatric *H. garnoti*, but less than that between Old World *H. bowringi* and New World *H. mabouia*.**

The Jurassic island of Nan Ao, on the Tropic of Cancer at the extreme eastern edge of Guangdong Province, China, remnant of the Zhe Min Old Land, has been above sea level millions of years longer than the nearby mainland or the better-known islands of Taiwan or Hong Kong (Lazell, 1999). Several species of amphibians and reptiles present on Nan Ao Island lack conspecific populations on the adjacent mainland but are apparently conspecific with remote populations to the north in interior upland China. This interrupted geographic pattern is termed *austro-boreal disjunction* (Lazell, 2004). *Gekko subpalmatus* is the most abundant of these. Originally using squamation characters we hypothesized that the population of apparent *G. subpalmatus* on Nan Ao Island was conspecific with *G. subpalmatus* from the widely disjunct main range of the species in the interior uplands of central China (Lazell et al., 1999). A protein electrophoretic assessment by Han et al. (2002) supported this view using as outgroups *Hemidactylus bowringi* from Nan Ao Island, the mainland of Guangdong Province, and Lantau Island of the Hong Kong Region.

We further hypothesized that the largely tropical species *G. chinensis* invaded intermediate lowland Guangdong and now occupies the gap between the putative *G. subpalmatus* disjuncts. Here we use DNA sequences from *G. chinensis* of tropical Lantau Island and subtropical interior mainland Guangdong (an intermediate zone between the *G. subpalmatus* disjuncts) to test this biogeographical notion and bolster the electrophoretic outcome of Han et al. (2002). Three species of *Hemidactylus* and *Gehyra mutilata* are used as outgroups to elucidate broader relationships; thus not just *G. subpalmatus* and *G. chinensis* but *Hemidactylus garnoti* and *H. mabouia* are added to the suite of 10 gekkonid species analyzed by Han et al. (2001) who used the same molecular technique to reveal some gekkonid relationships at specific and generic levels. These hypothesis tests should be just the first for many species pairs of similar

disjunctions and should eventually be compared to climatic and sea level data for South China and Nan Ao Island.

## MATERIALS AND METHODS

Lizards were hand-caught or noosed and fixed in 95% ethanol at 0°C or below. They were stored in this fluid at these temperatures for at least one week prior to being removed to room temperature and stored in 95% ethanol. All specimens were deposited at South China Normal University (SCNU) except Yale Peabody Museum (YPM) R15134, a *Hemidactylus garnoti* from Shui Hau, Lantau Island, Hong Kong (HK) whose liver alone returned to China and is now SCNU 060720. All geckos were captured in buildings or on walls at the following sites (abbreviated code): Tai Zhu Ao, Nan Ao, Guangdong Province, China, at sea level (NA); Song Bai Keng, Nan Ao, at 400 m (NA); Hu Yu, an islet off the northeast coast of Nan Ao (NA); Shui Hau, Lantau Island, Hong Kong Region, 10-20 m (HK); Dinghushan, Guangdong Province, China, 30 m (DHS); Chengdu, Sichuan Province, China, 450 m (SC); Guana Island, British Virgin Islands, 85 m (BVI); and Boot Key, Monroe County, Florida, USA, near sea level (FL). Our outgroup for all these geckos consisted of three species of skinks (Squamata: Scincidae): *Ateuchosaurus chinensis* and *Sphenomorphus indicus* from Nan Ao at 100 m and *Scincella modesta* from Lantau near sea level. Localities and numbers of specimens are indicated in Table 1 where abbreviations combine initials of a species with the locality codes above.

We extracted DNA from liver or muscle tissue using standard proteinase K digestion followed by phenol/chloroform extraction (Sambrook et al., 1989). The 400 bp nucleotide sequences of the 12S rRNA segment of mitochondrial DNA (mtDNA) were amplified with polymerase chain reactions (PCR). The primer sets were:

L109 (5'-AAAAAGCTTCAAACTGGGATTAGATACCCCACTAT-3') and

H1478 (5'-TGACTGCAGA GGGTGACGGGCGGTGTGT-3') (Kocher et al., 1989).

We ran PCR at least once for each specimen in a 50-μL PCR mixture that contained 100 ng template DNA, 25 μL 10×PCR master mix, and 7.5 μL of each primer (55 ng/μL). Each PCR sample was programmed on PTC100 or PTC200 with one cycle of denaturation at 95°C for 4 min, 35 cycles of denaturation at 95°C for 40 sec, annealing at 55°C for 40 sec, 72°C for 1 min, and a final extension at 72°C for 8 min. PCR products were stored at 4°C and later were purified and sequenced by Yinjun DNA Biotechnologies Company (Guangzhou, Guangdong, China) with an automated sequencer (Applied Biosystems ABI 377, Foster City, CA, USA).

The resultant nucleotide sequences were aligned with software ClustalX 1.81 (Thompson et al., 1997), and alignments were verified by eye. The number of mutations (variable sites) between DNA sequences of the different species was calculated with software MEGA2 (Kumar et al., 2001). Levels of inter- and intrapopulation genetic diversity were estimated by indices of haplotype diversity and by nucleotide diversity with software DNASP 3.14 (Rozas and Rozas, 1999). A phylogram was generated by neighbor joining (NJ) using software MEGA2 with 1000 replicates for bootstrapping; two additional phylograms were generated by minimum evolution (ME) and maximum parsimony (MP) also with 1000 replicates for bootstrapping for comparisons with the NJ phylogram.

## RESULTS

A total of 362-372 bp of nucleotide sequences were amplified (Table 2), with 147 sites variable in the total dataset (including outgroup), of which 135 were parsim-informpolymorphic sites (Pi) and 12 were singleton polymorphic sites (S). *Gekko subpalmatus* had the highest

number of variable sites among species and few of these (7%) were S sites, while *Hemidactylus bowringi* had none. *G. chinensis* had also a high number of variable sites and most of these (86%) were Pi sites.

Genetic distance between species (Table 3) was shortest (0.098) in the pair *Hemidactylus bowringi* and *H. garnoti*, and a close second (0.135) in the pair *Gekko subpalmatus* and *G. chinensis*. The lowest percentage divergence of base pairs (9.1%) was between the sympatric pair *H. bowringi* and *H. garnoti*. Percentage divergence of the parapatric pair *G. subpalmatus* and *G. chinensis* (20.6%) was similar to that of the allopatric pair *H. bowringi* and New World *H. mabouia* (18.8%) or that of the allopatric pair *H. garnoti* and *H. mabouia* (20.3%). Genetic distances between the three *Hemidactylus* species (0.098-0.241) bracketed the difference between the two *Gekko* species (0.135), indicating that relationships between *Hemidactylus bowringi* and *H. garnoti* were close, but relatively distant from *H. mabouia*.

Genetic distance within species was greatest in *Gekko subpalmatus* (0.046) among individuals from Nan Ao Island (Guangdong Province) and inland Chengdu (Sichuan Province). However, this within-species difference in *G. subpalmatus* was small compared to the between-species difference (Table 2: 0.135) in the pair *Gekko subpalmatus* and *G. chinensis*. In contrast, *Hemidactylus mabouia* (0.004) showed little variation among individuals from localities as far apart as Florida and the British Virgin Islands. Among individuals from five localities (Table 1) there was no variation in *H. bowringi* (0.000). Genetic distance among individuals within *G. chinensis* (0.031) between tropical Lantau Island (Hong Kong Region) and some from subtropical lowland Dinghushan (Guangdong Province) was comparable to that between *G. subpalmatus* disjuncts, supporting our hypothesis that the Dinghushan and Lantau populations were conspecific based on squamation.

Relationships of all nine species were expressed in the NJ tree (Fig. 1) and all species form distinct clades. These relationships were congruent with those in either ME or MP trees in that genetic distance among individuals between or within species of *Gekko* and *Hemidactylus* was consistent. The NJ tree placed *Gehyra mutilata* basal to both *Gekko* and *Hemidactylus*. The ME and MP trees placed *Gehyra mutilata* basal to *Gekko* or *Hemidactylus*, respectively. Han et al. (2001) also found the position of *Gehyra mutilata* equivocal. Populations of *G. subpalmatus* formed separated clades, but not those of *G. chinensis*: one specimen from Dinghushan was genetically similar to some from Lautau Island.

## DISCUSSION

The hypothesis of widely disjunct but conspecific populations of *Gekko subpalmatus* -- one on Nan Ao island and the other in upland central China -- is upheld. The minimum genetic distance found between species is greater than the maximum distance within species (0.098 in Table 3 versus 0.046 in Table 1). Our genetic data here are consistent with squamation and electrophoretic results by Han et al. (2002). *G. chinensis* in subtropical lowland Guangdong, geographically intermediate between the *G. subpalmatus* disjuncts, is also conspecific with the tropical Lantau Island population. All this agrees well with the notion of Lazell (2004) that the range of *G. subpalmatus* was continuous at glacial maximum, perhaps less than 25,000 years before present. However, lack of clearly defined geographical populations within *G. chinensis* (Fig. 1) may suggest recent time separation and continuous gene flow and that the high number of variable sites within *G. chinensis* may occur more at individual level than at population level but vice versus within *G. subpalmatus*.

Relationships of the three *Hemidactylus* species we included here conform to the larger

picture presented by Carranza and Arnold (2006) in that *H. bowringi* and *H. garnoti* are close relatives and *H. mabouia* is relatively distant. Failure to detect any variation within the populations of *Hemidactylus bowringi* spread over three islands and the mainland, spanning 475 km east-to-west and 125 km north-to-south, and from sea level to 400 m elevation, is notable. This perhaps reflects broad panmixis in this highly edificarian human commensal. Variation within *H. mabouia* is slight compared to that within either species of *Gekko* (Table 1), also probably reflecting its edificarian, human commensal niche. *H. mabouia* is a recent introduction to both the Virgin Islands and Florida, and possibly the entire western hemisphere (Townsend and Krysko, 2003; Lazell, 2005; but see Carranza and Arnold 2006).

*Gekko subpalmatus* is the first case of austro-boreal disjunction to be tested genetically; Lazell (2004) listed another dozen species that appear to present this pattern, not just between Nan Ao Island and interior upland China, but notably between the Florida Keys and north Florida and Georgia. Each pair of putative disjunct populations should be tested genetically and compared to geographically intermediate closely related taxa and eventually to climatic and sea level data for South China and Nan Ao Island to verify separation time.

## ACKNOWLEDGMENTS

We are indebted to Qin Chen, Kevin Enge, Mian Hou, Shanghua Huang, Jonathan Kolby, Kenneth Krysko, Peter Lynch, Jon Moore, Zhangpeng Su, Gregory Watkins-Colwell, and Jianli Xiong for specimens. This project was funded by The Conservation Agency, through a grant from the Falconwood Foundation, and Guangdong Science and Technology Foundation (No. 04010391).

## LITERATURE CITED

CARRANZA, S., AND E. N. ARNOLD. 2006. Systematics, biogeography, and evolution of *Hemidactylus* geckos (Reptilia: Gekkonidae) elucidated using mitochondrial DNA sequences. Molecular Phylogenetics an Evolution 38:531-545.

HAN, Y. L., X. XIAO, J. LAZELL, AND W. LU. 2002. Studies of the phenotypic character and consanguinity of isozymes in species and population. Hereditas, Beijing, 24:670-674 (in Chinese with English abstract).

HAN, D. M., K. Y. ZHOU, AND Y. Q. WANG. 2001. Phylogeny of ten species of gekkonid lizards (Gekkonidae: Lacertilia) inferred from 12S rRNA DNA sequences. Acta Zoologica Sinica 47(2):139-144 (in Chinese with English abstract).

KOCHER, T. D., W. K. THOMAS, A. MEYER, S. V. EDWARDS, S. PAABO, F. X. VILLABLANCA, AND A. C. WILSON. 1989. Dynamics of mitochondrial DNA evolution in animals: amplification and sequencing with conserved primers. Proceedings of the National Academy of Sciences, USA, 86:6169-6200.

KUMAR, S., K. TAMURA, I. B. JAKOBSEN, AND M. NEI. 2001. MEGA2: molecular evolutionary genetics analysis software. Bioinformatics 17:1244-1245.

LAZELL, J. 1999. The origins of the herpetofauna of the islands on the continental shelf of South China. In H. Ota, ed. Tropical Island Herpetofauna, pp. 79-96. Elsevier, Amsterdam.

LAZELL, J. 2004. Austro-boreal disjunctions: a remarkable biogeographic pattern illustrated by Nan Ao Island, Guangdong, China. Journal of Biogeography 31:1261-1265.

LAZELL, J. 2005. Island: Fact and Theory in Nature. University of California Press, Berkeley, CA.

LAZELL, J., J. KOLBY, Y. LIN, D. ZHUANG, AND W. LU. 1999. Reptiles and amphibians from

Nan Ao Island, China. Postilla 217:1-18.

ROZAS, J., AND R. ROZAS. 1999. DNASP version 3.0: an integrated program for molecular population genetics and molecular evolution analysis. Bioinformatics 15:174-175.

SAMBROOK, J., E. F. FRITSCH, AND T. MANIATIS. 1989. Molecular Cloning. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY.

TOWNSEND, J. H., AND K. L. KRYSKO. 2003. The distribution of *Hemidactylus* (Sauria: Gekkonidae) in northern peninsular Florida. Florida Scientist 66(3):204-208.

Fig. 1. Rooted neighbor joining (NJ) phylogram of 12S rRNA segment of mtDNA among nine species of lizards with bootstrap values on branches where abbreviations for species and locality are from Table 1 and number of individuals is in parentheses.

(NQ, RT) SOUTH CHINA NORMAL UNIVERSITY, COLLEGE OF LIFE SCIENCE, GUANGZHOU, GUANGDONG 510631, CHINA; qingn@scnu.edu.cn. (WL, JL) THE CONSERVATION AGENCY, 6 SWINBURNE STREET, JAMESTOWN, RI 02835, USA; hq@theconservationagency.org.

TABLE 1. WITHIN-SPECIES GENETIC DISTANCE FOR FOUR SPECIES OF HOUSE GECKOS BASED ON THE 12S RRNA SEGMENT OF MTDNA. Number and locality of specimens are indicated for all nine species used in analyses.

| Species | Locality | Specimen | Abbreviation |
|---|---|---|---|
| *Gekko chinensis* | Guangdong: Dinghushan | 4 | GcDHS |
| | Hong Kong: Lantau | 3 | GcHK |
| *Gekko subpalmatus* | Guangdong: Nan Ao | 3 | GsNA |
| | Sichuan: Chengdu | 3 | GsSC |
| *Hemidactylus bowringi* | Guangdong: Nan Ao: Tai Zhu Ao | 2 | HbNA |
| | Guangdong: Nan Ao: Song Bai Keng | 1 | HbNA |
| | Guangdong: Nan Ao: Hu Yu | 2 | HbNA |
| | Hong Kong: Lantau | 1 | HbHK |
| | Guangdong: Dinghushan | 3 | HbDHS |
| *Hemidactylus garnoti* | Hong Kong: Lantau Island | 1 | HgHK |
| *Hemidactylus mabouia* | USA: Florida | 3 | HmFL |
| | BVI: Guana Island | 3 | HmBVI |
| *Gehyra mutilata* | Hong Kong: Lantau Island | 1 | GmHK |
| *Ateuchosaurus chinensis* | Guangdong: Nan Ao | 1 | AcNA |
| *Scincella modesta* | Hong Kong: Lantau Island | 1 | SmHK |
| *Sphenomorphus indicus* | Guangdong: Nan Ao | 1 | SiNA |

TABLE 2. NUMBERS OF BASE PAIRS IN THE 12S RRNA SEGMENT OF MTDNA FOR SIX SPECIES OF HOUSE GECKOS. C stands for conserved site of a base pair, V for variable site, Pi for parsim-informpolymorphic variable site, S for singleton polymorphic variable site, and / for not applicable due to lack of specimens for calculation.

| Species | C | V | Pi | S | Total |
|---|---|---|---|---|---|
| *Gekko chinensis* | 342 | 29 | 25 | 4 | 372 |
| *Gekko subpalmatus* | 339 | 31 | 28 | 3 | 370 |
| *Hemidactylus bowringi* | 362 | 0 | 0 | 0 | 362 |
| *Hemidactylus garnoti* | / | / | / | / | 366 |
| *Hemidactylus mabouia* | 361 | 2 | 2 | 0 | 362 |
| *Gehyra mutilata* | / | / | / | / | 363 |
| Total | 230 | 147 | 135 | 12 | 382 |

TABLE 3. BETWEEN-SPECIES GENETIC DISTANCE AND PERCENTAGE DIVERGENCE OF BASE PAIRS IN PARENTHESIS AMONG SIX SPECIES OF HOUSE GECKOS BASED ON THE 12S RRNA SEGMENT OF MTDNA.

| | Species | 1 | 2 | 3 | 4 | 5 |
|---|---|---|---|---|---|---|
| 1 | *Gekko chinensis* | | | | | |
| 2 | *Gekko subpalmatus* | 0.135 (20.6) | | | | |
| 3 | *Hemidactylus bowringi* | 0.239 (24.0) | 0.267 (25.5) | | | |
| 4 | *Hemidactylus garnoti* | 0.264 (26.0) | 0.257 (26.0) | 0.098 (9.1) | | |
| 5 | *Hemidactylus mabouia* | 0.308 (28.1) | 0.318 (28.4) | 0.233 (18.8) | 0.241 (20.3) | |
| 6 | *Gehyra mutilata* | 0.300 (28.9) | 0.300 (27.9) | 0.349 (26.3) | 0.335 (25.3) | 0.368 (27.1) |

Fig. 1. Rooted neighbor joining (NJ) phylogram of 12S rRNA segment of mtDNA sequences among nine species of house geckos with bootstrap values on branches where abbreviations for species and locality are from Table 1 and number of individuals is in parentheses.